Remarks

Claims 11-14, 16-18, 20-23 and 28 are pending in the application. The Examiner's detailed review of the application and consideration of the Applicant's previous amendment are noted with appreciation.

Claim Rejections – 35 U.S.C. § 102

The claims have been rejected under 35 U.S.C. § 102 as allegedly anticipated by Hodgson (U.S. Pat. No. 6,410,220). Hodgson describes a method for directing the self assembly of gene fragments to produce a gene, gene vector or large nucleic acid molecule. According to Hodgson's method, at least three different gene fragments having known restriction sites are produced by PCR. The amplified fragments are digested with the appropriate restriction endonucleases to create single-stranded overhangs that are complementary to a specific single-stranded overhang on another fragment. The amplified and digested fragments are then combined in a ligation reaction to create a directionally ordered molecule.

Ligating a DNA insert and a DNA vector
in the presence of a DNA compaction agent

This application includes independent claims 11 and 28. Each independent claim recites the step of producing circularized recombinant nucleic acids by ligating a DNA insert and a DNA vector in the presence of a DNA compaction agent. The Examiner has taken the position that Hodgson describes the ligation of a DNA insert and a DNA vector in the presence of a DNA compaction agent. The Official Action indicates that the passage of col. 23, lines 17-27, of Hodgson is particularly relevant. However, that passage is silent with respect to the presence of a DNA compaction agent while ligation is performed. Therefore, the Examiner points to the

passage at col. 23, lines 49-52, which states that "[a]nother method is to add a DNA condensing reagent (dendrimers, polycations [such as polyethyleneamine] histones or liposomes) directly to the ligation reaction."

The Examiner has taken the position that the passage describing the addition of condensing reagent directly to the ligation reaction milieu indicates that the ligation itself must be carried out in the presence of the condensing reagent. The Applicant respectfully submits that the condensing reagent is added to the reaction milieu after ligation is complete. When read out of context the passage is, at best, ambiguous as to whether the condensing reagent is added before or after addition of the ligase, as evidenced by the Examiner's and Applicant's disagreement on this point. The above-quoted passage mentions the addition of a DNA condensing reagent, but does not explicitly indicate whether the condensing reagent is added to the reaction before, during or after the ligation is performed. Therefore, the passage must be read in context, while considering the general knowledge of one skilled in the art, to determine if the ligation reaction was performed in the presence of the condensing reagent.

The paragraph of Hodgson mentioning the addition of a DNA condensing reagent is directed to a discussion of special considerations applicable to large vectors. It is noteworthy that the large vectors discussed are the product of the ligation, and not the fragments to be assembled in the ligation reaction. The paragraph discussing the special circumstances confirms this point by indicating that it is desirable to avoid shearing "once large segments have been joined by ligation". In the paragraph, Hodgson provides two suggestions to avoid shearing "once large segments have been joined together".

The first suggestion is to add the transfection agent and then the cells to be transfected directly to the ligation reaction, to avoid the need to physically move the ligated molecules. The

second suggestion is to add a DNA condensing reagent directly to the ligation reaction and then move the DNA by pipette after it has been condensed. It is noteworthy that both suggestions use the phrase "directly to the ligation reaction" in describing the components to be added. One skilled in the art would consider the following points in interpreting the meaning of the phrase "directly to the ligation reaction" as used in Hodgson.

One skilled in the art would understand the manner in which DNA is classically purified after enzymatic intervention. DNA is generally purified by precipitation, which involves deproteinization of the DNA using a phenol-chloroform mixture, transfer of the upper phase into a fresh tube, precipitation of the DNA by alcohol, centrifugation, washing, drying, and re-suspension of the DNA in solution. The DNA can also be purified, after deproteinization, by extraction from a gel of agarose or acrylamide, or by filtration using suitable filters. All of these techniques involve pipetting the solutions or DNA suspension, which can damage the DNA by shearing (breaking one or both of the two chains). If the DNA is of a large size, it tends to break more readily because the natural breaking points multiply with the length. The relevant Hodgson paragraph is concerned with such shearing of the large, assembled vectors.

As noted above, the phrase "directly to the ligation reaction", which Hodgson uses to describe both the addition of transfection agent (according to the first suggestion) and the addition of condensing reagent (according to the second suggestion), can be ambiguous when taken out of context. It could mean that the reagents are added at the same time as the various fragments and the ligase, as contended by the Examiner. Alternatively, it can mean that the reagents are added to the products of the reaction of ligation, *i.e.*, the resultant ligated DNA, as contended by the Applicant. Only the context in which the phrase appears can make it possible to determine which interpretation is the correct one.

As explained above, the context in which the phrase "directly to the ligation reaction" must be considered is the passage in Hodgson that suggests two methods to avoid shearing large DNA molecules "once the segments have been joined by ligation". *See,* col. 23, line 41. The first suggestion is the addition of a transfection agent (dendrimeres, liposomes or others) "directly to the ligation reaction", followed by the addition of cells to be transfected. Hodgson specifies that these steps are performed on the already ligated DNA at col. 23, lines 47-49 as follows:

> This, or a similar method avoids the need to physically move the ligated DNA, and thus prevents shearing. (emphasis added)

The following passage at col. 25, lines 32-36, in Hodgson confirms that the transfection agents are added "directly to the ligation reaction" after the ligation has already taken place:

> The vector part and the helper virus part are combined ... after ligation has been performed separately on each fragment set. The superfect protocol (Qiagen) is used to transfect the vector part and the helper part into the helper cells. (emphasis added).

Based on the above passage, it is clear that distinct ligations of the vector part and the "helper" part are carried out separately before the addition of the transfection agent. After ligation, both ligated DNA products are combined. The transfection agent is subsequently added to the mixture of both ligated DNA products, and not added to each reaction mixture prior to ligation.

The second suggested method to avoid shearing is also performed on ligated DNA. According to the second method, the component that is added "directly to the ligation reaction" is "a DNA condensing reagent". Here, use of the term "condensing" introduces yet another ambiguity into the Hodgson description. "Condensing" could mean compaction. Alternatively, "condensing" could mean thickening until solidification, *i.e.*, precipitation. According to the

New Webster's dictionary (1981) the word "condense" means to make more dense or compact; or to reduce, as a gas or vapor to the condition of a solid or liquid.

To evaluate the nature of Hodgson's condensing reagents, it is useful to consider the exemplary condensing reagents listed therein. According to Hodgson, histones are used interchangeably with liposomes, dendrimers, and polycations, such as PEI. Because these are common transfection agents, one skilled in the art would understand that the "condensing reagent" is employed as a transfection agent having precipitation properties. DNA precipitation is generally desirable in transfection, to facilitate the transfer of the DNA molecules through the pores of the cells to be transfected. In fact, the majority of the transfection agents are DNA precipitating agents. (It is likely that there is also DNA precipitation when the transfection agent is added to the ligated DNA in the first method, but it is not an important point for that method.)

Continuing the evaluation of Hodgson's second suggested method to avoid shearing, the context of the passage indicates that the condensing reagent is added in an amount sufficient for DNA precipitation. Indeed, it is then adequate to hang the ligated DNA, which is complexed with the condensing reagent at the end of a rod (the pipette, which the investigator conveniently has at his or her disposal) to bring it into contact with the cells to be transfected (or to be infected if the ligated DNA is a virus). The condensing agent acts also as a transfection agent. Thus, the pipetting of large DNA molecules, which tends to shear the DNA, is avoided and the number of handling steps is reduced because instead of precipitating with alcohol, with all of the necessary purification steps, the DNA is precipitated directly by the transfection agent.

The use of the second method proposed by Hodgson, which the skilled artisan would understand to involve transfection/condensing reagent, may be used instead of the first method

where circumstances warrant. For example, it can be useful to move the ligated DNA if the cells cannot be moved, such as if the cells are immobilized on a support. For these reasons, one skilled in the art would understand that the Hodgson "condensing reagent" is added for the purpose of precipitating the DNA vectors after DNA ligation has been completed so that the DNA can be transferred without shearing, an advantage in any circumstance where it is impractical or undesirable to move the cells to be transfected. As such, it is clear that the "condensing reagent" is added after ligation has been performed.

Another passage in Hodgson, at col. 24, lines 40-43, further confirms that when the second method to avoid shearing is used, the condensing reagent is added after ligation is complete:

> To prevent damage during handling, the minichromosome DNA can be condensed with polycations, adenovirus particles, dendrimers, histones, or liposomes prior to transfection, similar to larger viral vectors.

The passage reproduced above specifically refers to line 50 of column 23. The minichromosome is the product of the ligation. It is the minichromosome itself that is precipitated, and not the fragments which were used to assemble it. Thus, it is clear that in the context of adding a condensing reagent, the expression "directly to the ligation reaction" means addition after the ligation is complete.

In sharp contrast, this application claims a method that includes the step of producing circularized recombinant nucleic acid by ligating a DNA insert and a DNA vector in the presence of a DNA compaction agent. As explained in the Background section of the application, recombinant vector construction includes the step of inserting a DNA fragment into a vector and then reclosing the vector to form a circularized molecule. The reclosure step requires that the ends of the DNA molecule be close to one another. Thus, the probability of conjunction of the ends decreases as the length of the vector increases. This invention improves the probability of

reclosure for large vectors by performing ligation in the presence of a DNA compaction agent, which brings the ends closer together. Thus, according to this invention, the DNA compaction agent is present during the ligation.

Because Hodgson does not describe or suggest the step of producing circularized recombinant nucleic acids by ligating a DNA insert and a DNA vector in the presence of a DNA compaction agent, it is requested that the rejection of claims 11-14, 16-18, 20-23 and 28 under 35 U.S.C. § 102 be reconsidered and withdrawn.

DNA compaction agent is present at a concentration sufficient to allow flexibitlity of said DNA insert

Each of the independent claims also recites that the DNA compaction agent is present at a concentration sufficient to allow flexibitlity of the DNA insert. This feature also distinguishes the invention from Hodgson. Because Hodgson is concerned with precipitating the DNA after ligation is complete, Hodgson provides no description, suggestion or motivation to provide the "condensing reagent" in a concentration that would allow the DNA to remain flexible. To the contrary, Hodgson's condensing reagent, which is intended to precipitate the DNA, would be more likely to cause rigidity.

DNA precipitation is not an object of the process of this invention, as it is in the second suggested method of Hodgson. Instead, DNA precipitation could cause the fragments to become rigid, which would impede their ligation. Therefore, according to this invention, the amount of compaction agent is carefully selected to provide the benefits of the invention (bringing the ends of the vector close together to facilitate reclosure), while not causing excessive loss of flexibility or precipitation.

In this last respect, it should be noted that it is not only a question of the flexibility of the naked DNA, but of the flexibility of the continuous complex of DNA and compaction agent. Thus, where histones are the compaction agent, the invention concerns the flexibility of formed chromatin fiber. The flexibility to be considered is that of the entire recombinant DNA molecule, as well as the insert. The amount of compaction agent present during ligation impacts this flexibility. Too much compaction agent would make the complexed DNA too rigid for effective ligation, while too little would not provide the optimum benefit. Because Hodgson fails to contemplate the presence of a "condensing reagent" during ligation, Hodgson naturally provides no description or suggestion to provide it in a concentration that allows flexibility. The carefully selected concentration of compaction agent not only distinguishes this invention from Hodgson, but also sets the invention apart from conventional ligation experiments, where only the flexibility of the naked DNA molecule is generally of significance.

Thus, the § 102 rejection of claims 11-14, 16-18, 20-23 and 28 should also be reconsidered and withdrawn because Hodgson does not describe or suggest the provision of a DNA compaction agent at a concentration sufficient to allow flexibility of the DNA insert.

Claim Rejections – 35 U.S.C. § 103

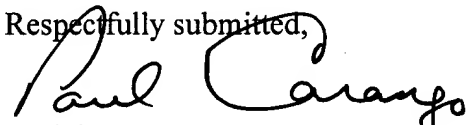
Claims 20 and 21 have been rejected under 35 U.S.C. § 103 as allegedly obvious over the combination of Hodgson and Nagaki. Claims 20 and 21 depend from claim 11 and further define the concentration of compaction agent to be used. Nagaki is cited for the proposition that it allegedly describes a particular concentration of compaction agents in the form of HMG. Although Nagaki mentions that HMG "bends" DNA, it fails to describe or suggest the flexibility of DNA complexed with a compaction agent, as in this invention. Also, Nagaki does not suggest the use of a compaction agent in ligating a DNA insert into a DNA vector to make a circularized

recombinant molecule. Moreover, because claims 20 and 21 are dependent on claim 11 and because Nagaki has not been cited as curing the other previously discussed deficiencies of Hodgson with respect to independent claim 11, claims 20 and 21 are also patentable for at least the reasons set forth above.

Conclusion

For the reasons explained above, it is respectfully requested that all of the rejections and objections set forth in the Official Action be reconsidered and withdrawn. The Applicant submits that the Application is now in condition for allowance, which is respectfully requested. If the Examiner believes that corrections as to matters of form or other minor amendments would advance the application, the Examiner is invited to telephone the Applicant's undersigned representative.

Respectfully submitted,



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